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Optimization of Pectin Extraction from *Nephrolepis biserrata* Leaves Using Response Surface Methodology

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Abstract

A central composite design was employed to optimize the extraction of pectin from *Nephrolepis biserrata* leaves. The independent variables were pH (1.5 to 2.5), extraction time (60 to 120 minutes) and temperature (60°C to 100°C). The combined effect of these variables on yields of pectin was investigated. The results showed that the yield of extracted pectin ranged from 3.76% to 8.50% (w/w, based on dry weight of *Nephrolepis biserrata* leaves). The optimum condition for the yield of pectin extraction was predicted at pH (1.5), extraction time (76.25 minutes) and temperature (100°C). Under the optimum condition, the actual pectin yield was 8.18%, which was below the predicted extraction condition of 8.316 %. The characteristics were 47.52% galacturonic acid and 83.71% degree of esterification.

INTRODUCTION

Pectin is one of the major components of primary cell wall and it is generally thought to account for about one third of all primary cell wall macromolecules. The middle lamella and the primary cell wall of higher plants contain complex heteropolysaccharide called pectin [1]. Pectins are group of polysaccharides consisting mostly of D-galacturonic acid [2]. Some of the carboxylic groups of galacturonic acid molecules in the pectin chains are methyl esterified and the percentage of esterified groups is expressed as DE (degree of esterification). Depending on the degree of esterification, pectin is divided into two majors groups; higher-ester pectin with DE more than 50%, and low-ester pectin with DE lower than 50% [3].

Pectin is extensively utilized in food processing, especially for the conversion of low grade fruits into good quality products like jam, jelly, marmalade and candies [4,5]. The yield and quality of pectin depends mostly upon the source, as well the method employed for extraction of pectin [6]. Mollea *et al.,1* conducted a preliminary study in the optimization of pectin extraction from cocoa husks, where the highest yield of pectin was 8.0 % at pH 2.5 and extraction time of 1 hour[7].

At the moment, researches on extraction of pectin from lignocellulose sources other than fruits and vegetables are rarely performed, and the research on the extraction of pectin from *Nephrolepis biserrata* has not been studied. Therefore, it is necessary to study and investigate on the utilization of *Nephrolepis biserrata.* Based on a previous test (fractionation) on *Nephrolepis biserrata,* lignocellulose content of 34.27%, 1.78% of hemicellulose, 20.66% of cellulose and 43.29% of lignin were determined by using 0.5 M sulfuric acid at 100°C for 4 h. *Nephrolepis biserrata* (Sw) is very easy to find in Malaysia and in other agrarian countries. *Nephrolepis biserrata* is rich with crude protein content, where the highest content can be found in its leaf (Yeoh

and Wee. 1998)[8]. Therefore, *Nephrolepis biserrata* can be used as an alternative to produce pectin as it is cheap and easily obtainable.

Material and Methods

Sample

Fresh *Nephrolepis biserrata* leaves were collected within the vicinity of Universiti Teknologi Malaysia.

Methods

Pectin Extraction

The extraction procedure was according to the the Rehman *et al*. method, with slight modification [4]. A total 5 g of *Nephrolepis biserrata* leaves was dried in an oven at 50°C – 60°C until a constant weight was achieved. Dry weight was recorded for pectin yield determination. The dried *Nephrolepis sp* leaves were blended and kept at a substrate to water ratio of 1:40. The desired pH of the mixture was adjusted with 0.5 N sulfuric acid for pH (1.5-2-2.5), and then incubated at the temperature (60°C-80°C-100°C) for different extraction times (60-90-120 minutes) with frequent stirring. After incubation, the contents were filtered through filter paper and pectin from the filtrate was precipitated with ethanol of 95% concentration. The obtained pectin was dried in an oven at 40°C until a constant weight was achieved and ground finely to analyze its chemical quality characteristic. The yiled was calculated as the weight of dried pectin (g) per 100 g of dried *Nephrolepis biserrata* leaves.

Experimental Design and Statiscal Analysis

The optimization of yield of pectin extraction was carried out using responce surface methodology (RSM). The variables used were pH (A), extraction time (B) and temperature (C), and the values of the variables were coded as –α, -1, 0, +1, +α (low, basal, high) (Table 1).

Table 1. Range of variables and their coded levels

	Coded value				
Independent	–α	-1	0	+1	+α
A pH	1.16	1.5	0	2.5	2.84
B Extraction time (h)	39.55	60	90	120	140.45
C Temperature (°C)	46.36	60	80	100	113.64

The effect of the three variable levels on pectin extraction ratio was studied to determine the optimum combination of the variables using the central composite design (CCD) in RSM [9]. Experimental data were analyzed to fit the following regression model with interaction terms are given below:

$$Y = X_0+X_1A+X_2B+X_3C+X_{11}A2+X_{22}B2+X_{33}C2+X_{12}AB+X_{13}AC+X_{23}BC \quad (1)$$

where Y is the yield of pectin % (w/v), X_0, X_1, X_2,...X_{23} represent the estimated regression coefficients, X_1, X_2, X_3 represent the linear effect, X_{11}, X_{22}, X_{33} represent the quadratic effect and X_{12}, X_{13}, X_{23} represent the cross product coefficient, while A, B and C repressent the variables studied (pH, extraction time and temperature). The statistical analysis was performed by using Design Expert software.

Characterization Pection Extracted from *Nephrolepis biserrata* Leaves at Optimum Condition

The determination of galacturonic acid content and the degree of esterification were conducted according the method described [10].

RESULT AND DISCUSSION

Results of RSM

The regression equation representing the relationship between pectin extraction ratio and the test varibles derived from RSM is as follows:

$$Y = 6.91 - 0.88*A + 0.17*B + 0.63*C - 0.35*A^2 - 0.59*B^2 - 0.32*C^2 + 0.21*A*B - 0.31*A*C - 0.51*B*C \quad (2)$$

Y is the equation terms of the coded factors. The ANOVA in this case confirmed the adequacy model. When the Prob>F value is less than 0.05, it shows that the term is significant. The value of R^2 for the yield was 9.270. This indicates that 92.7% of the total variation was explained by the model. The results of analysis of variance (ANOVA) for the yield are given in Table 2. The ANOVA revealed that the model adequately fit the experimental data for yield.

By analyzing and preparing the pertinence and significance of every variable, the optimized extraction condition was pH 1.5, extraction time of 76.25 minutes and temperature of 100°C, and the expected yield was 8.18 %. By performing the pectin extraction under recommended extraction at optimum condition, is the yield was 8.316 %. The 3-dimensional response surface plot for yield is given in Figure 1, showing the effect of (a) pH and extraction time on yield (temperature of 80°C), (b) pH and temperature (extraction time of 90 minutes) and, (3) extraction time and temperature (pH of 2.0).

Table 2 The results of analysis of variance (ANOVA) for yield of pectin

Source	Sum of Square	Mean Square	Standard Error	F-value	p-value
Model	26.60	2.96	-	14.11	0.0001*
A pH	10.53	10.53	0.12	50.29	<0.0001
B Extraction	0.38	0.38	0.12	1.80	0.2095
C Temperature	5.34	5.34	0.12	25.49	0.0005
A^2	1.79	1.79	0.12	8.56	0.0151
B^2	5.01	5.01	0.12	23.92	0.0006
C^2	1.52	1.52	0.12	7.24	0.0226
AB	0.34	0.34	0.16	1.62	0.2313
AC	0.79	0.79	0.16	3.76	0.0812
BC	2.11	2.11	0.16	10.08	0.0099
Lack of fit	1.69	0.34	-	4.20	0.0706
Intercept	6.91	-	0.19	-	-

Note:* p-value <0.005 is significant

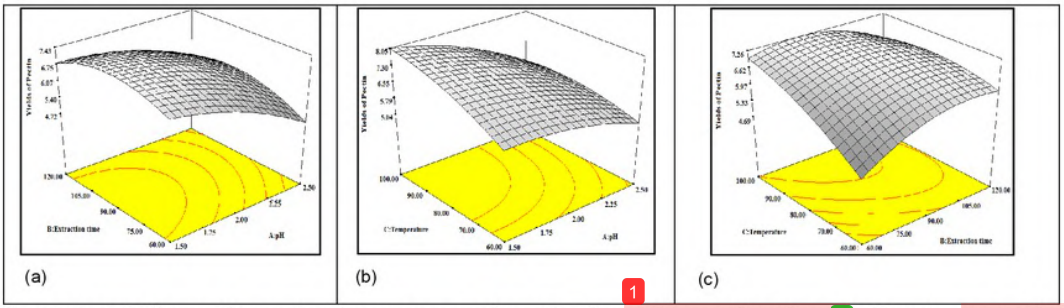

Figure 1. (a) Three–dimensional response surface plot for yield as a function of pH and extraction time (temperature of 80°C), (b) three–dimensional response surface plot for yield as a function of pH and temperature (extraction time of 90 minutes), and (c) three–dimensional response surface plot for yield as a function of extraction time and temperature (pH 2.0).

Results of Characterization of Pectin Extracted from *Nephrolepis biserrata* Leaves at Optimum Condition

The results of characterization of pectin from *Nephrolepis biserrata* leaves at optimum condition using RSM showed there was 47.52% of galacturonate acid , while the degree of esterification was 83.71%.

CONCLUSION

Based on the RSM results and discussion, it can be said that pH, extraction time and temperature are important factors and they are statistically significant in the extraction process, as well as play an important role to maximize the yield of pectin from *Nephrolepis biserrata* leaves. By analyzing and preparing the pertinence and significance of every variable, the optimized extraction condition was pH 1.5, extraction time of 76.25 minutes and temperature of 100°C. By performing pectin extraction under the recommended extraction optimum condition, the yield was 8.18%, which was belom the predicted extraction condition of 8.316%. Thus, the optimization extraction technology is feasible. The resulting degree of esterification was higher than 50%, which indicated *Nephrolepis biserrata* leaves is suitable as an alternative source of pectin for food industry.

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